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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MAY 5 1995

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MEMORANDUM

SUBJECT: Consideration for Section 3(c)(7)(B) Conditional Unlimited Registration of the Plant-pesticide *Bacillus thuringiensis* subspecies *tenebrionis* (*B.t.t.*) Colorado Potato Beetle (CPB) Control Protein (EPA File Symbol 524-474)

. . . . . DECISION MEMORANDUM . . . . . .

FROM: Janet L. Andersen, Acting Director
Biopesticides and Pollution Prevention Division

TO: Daniel M. Barolo, Director
Office of Pesticide Programs

I. ISSUE

Should the Agency: (1) conditionally amend the existing FIFRA §3(c)(5) registration for limited plant propagation use to permit an additional use of the product in food and feed potatoes pursuant to FIFRA §3(c)(7)(B); and (2) amend the existing plant propagation registration issued under FIFRA §3(c)(5) by converting it to a FIFRA §3(c)(7)(B) "conditional" registration for all uses and by eliminating the existing terms and conditions of that registration? The active ingredient in this pesticide product is the *Bacillus thuringiensis* (*B.t.*) CryIII(A) delta endotoxin and the genetic material necessary for its production in potatoes (referred to by Monsanto as *Bacillus thuringiensis* subspecies *tenebrionis* (*B.t.t.*) Colorado Potato Beetle (CPB) Control Protein). "Genetic material necessary for production" means the CryIII(A) gene and its regulatory regions. "Regulatory regions" means genetic material that control expression of the gene such as promoters, terminators, and enhancers. The limitations currently placed upon the use of the product include but are not limited to the acreage which may be planted, the

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duration of the registration, geographic areas where the product may be used, and post-harvest agricultural practices.

II. SUMMARY AND REGULATORY BACKGROUND

Monsanto Company of St. Louis Missouri, has submitted an application for registration and a petition requesting exemption from the requirement of a tolerance for delta endotoxin from the bacterium *Bacillus thuringiensis* subspecies *tenebrionis* (*B.t.t.*) and the genetic material necessary for its production (*B.t.* CryIII(A) delta endotoxin). The announcement of receipt of the application for registration of a new active ingredient and the petition for exemption from the requirement of a tolerance were published in the Federal Register on December 8, 1993.

The neomycin phophotransferase II resistance marker gene (inert ingredient) was granted an exemption from the requirement of a tolerance in or on all raw agricultural commodities on September 28, 1994.

The Agency published its proposed position on the regulation of pesticidal substances produced in plants (59 FR 60496, November 23, 1994). In the proposal, the Agency would designate the pesticidal substances produced by plants as plant-pesticides. In addition, the Agency issued proposed regulations that define certain categories of plant-pesticides that would be exempt from regulation under FIFRA and FFDCA. Plant-pesticides not exempt would be subject to regulation. The *Bacillus thuringiensis* delta endotoxins are examples of plant-pesticides that would be regulated under the proposal.

On March 17, 1995, the Agency issued a limited registration that allows Monsanto to produce plant propagation materials (i.e., seed potatoes), but that is limited in scope and duration. The following items were terms and conditions of that registration. These terms and conditions would be removed when the amended registration is accepted:

1. Monsanto shall enter into a written agreement with each of its cooperators that requires the cooperator to comply with all the terms and conditions of this registration. "Cooperator" means any person who has been granted permission from Monsanto to use the pesticide product on property owned or controlled by that person.

2. Monsanto shall be liable under the Federal Insecticide, Fungicide and Rodenticide Act for any action of a cooperator that does not comply with the terms and conditions of this registration.

3. Plants that contain the pesticide product may be grown on no more than a total of 8,186 acres. Plants that contain the

pesticide product may only be grown in the states of: Colorado, Idaho, Maine, Michigan, Minnesota, Montana, Nebraska, New York, North Dakota, Oregon, Washington, and Wisconsin.

4. The registration of this pesticide product will automatically expire on midnight of March 15, 1996, unless EPA extends the registration.

5. No plants or plant propagation materials that contain the pesticide product may be sold or distributed, except that such plants and materials may be distributed between Monsanto and its cooperators and may be distributed for storage under Monsanto's control.

6. All plant propagation materials produced by Monsanto and its cooperators that contain the pesticide product must be either destroyed or securely stored for future plantings, research, or used for additional plant propagation material pursuant to the conditions of this registration.

7. All plants and plant materials that remain in the field after harvest shall be destroyed by tilling into the soil.

8. After this registration has expired, no plants or plant propagation materials that contain the pesticide product may be planted, grown, or harvested.

A meeting of a subpanel of the Federal Insecticide, Fungicide, Rodenticide Act Science Advisory Panel (SAP) was held on March 1, 1995, to allow for external scientific peer review and public participation in the decision process. The final SAP report does not identify any issues that would preclude the amendment of the limited plant propagation registration to allow conditional registration of the product and issuance of the exemption from the requirement of a tolerance.

The Biopesticides and Pollution Prevention Division (BPPD) has evaluated the data submitted by Monsanto and, based on these data and other relevant information, believes that the product will perform its intended function and does not expect any unreasonable adverse effects to humans, nontarget organisms, or the environment from the use of this product. BPPD scientists have reviewed the information submitted with respect to health effects, and these data show that the product will be digested like any other protein and genetic material and will have no effects on human health. Likewise, the data submitted for ecological effects have identified no hazards to non-target organisms. The benefits data have been reviewed and the product has been found to be in the public interest. The submitted data show potentially substantial benefits from the use of the product. Colorado potato beetle is extremely difficult to control, and has been the subject of FIFRA Section 18 Emergency

Exemptions in past years. The *B.t.* CryIII(A) delta endotoxin not only exhibits superior pest control and potential economic savings to growers, but also is a substitute for more hazardous registered organophosphate, carbamate, and pyrethroid products.

The amended registration, if granted, will be conditional under section 3(c)(7)(B) of FIFRA. The Agency is requiring non-target studies for earthworms and *Collembola*, along with validation of the honeybee larval study, as conditions of the registration. These studies were not listed as a requirement for this active ingredient prior to the date of the data submission by Monsanto. Effects to these non-targets are not anticipated, based upon available information and the results reported in the honeybee larval study.

PUBLIC COMMENTS

In addition to the Federal Register notice announcing the receipt of the application, the Agency additionally announced the availability of the data in support of this application on January 25, 1995 and February 8, 1995, along with an additional public comment period. Twenty-five individuals or organizations provided written comment on the proposed registration. Most written comments were positive in nature, noting the potential benefits of the plant-pesticide. No risk concerns relating to effects on humans or nontarget sites were identified in any of these comments. A few commenters expressed concerns regarding emergence of resistance to the pesticide in the target pest. These comments are addressed in section G, below. Comments regarding the SAP meeting are also discussed in that section.

III. SCIENCE ASSESSMENT

The discussion that follows summarizes BPPD's review of the data available to the Agency on this product. A more detailed discussion of this assessment is provided in the Data Evaluation Records for the studies summarized below.

A. RESIDUE CHEMISTRY DATA

Residue chemistry data were not required, because of the lack of toxicity to this active ingredient. The active ingredient in this product is derived from *Bacillus thuringiensis* subspecies *tenebrionis*. In not requiring residue chemistry data for this product, EPA has a taken a position similar to the one it takes regarding the submission of residue data for the microbial *Bacillus thuringiensis* products from which this plant-pesticide was derived. [See 40CFR Sec. 158.740(b).] For microbial products, residue data are required only when Tier II or III toxicology data are required. The appropriate kinds of studies for this plant-pesticide are based upon like those in Tier I, not Tiers II or III. Submitted data indicated that the product is of low

mammalian toxicity and supported the conclusion that the kinds of studies required in Tier II or III were not appropriate. Therefore, no residue data were required in order to grant an exemption from the requirements of a tolerance for the *Bacillus thuringiensis* CryIII(A) delta endotoxin protein and the genetic material necessary for its production in potato.

B. PRODUCT ANALYSIS

Monsanto submitted information which adequately described the plant pesticidal substance, *B.t.* CryIII(A) delta endotoxin as produced in potato. Because it would be difficult, or impossible, to extract sufficient biologically-active toxin from the plants to perform toxicology tests, Monsanto used a-endotoxin produced in bacteria. Product analysis data were submitted to show that the microbially expressed and purified *B.t.* CryIII(A) delta endotoxin is sufficiently similar to that expressed in the plant to be used for mammalian toxicological purposes.

1. Molecular Characterization of CPB Resistant Russet Burbank Potatoes Equivalence of Microbially-Produced *B.t.t.* Protein - The relative size and number of copies of the DNA inserted into potatoes was demonstrated with endonuclease digested chromosomal DNA from field grown potato plants southern blotted with the entire introduced plasmid PV-STBT02 as the probe. These southern blots provided information about the number of copies of introduced DNA, the lack of significant amount of DNA introduced outside the border regions and integrity of the introduced DNA near the endonuclease cut site. These results indicate that only the DNA necessary to produce the CryIII(A) delta endotoxin were introduced into the plant.

2. Equivalence of Microbially-Produced and Plant Produced *B.t.t.* Protein also called Colorado Potato Beetle Active Protein from *Bacillus thuringiensis* subsp. *tenebrionis* - Microbially produced delta endotoxin from the Cry III(A) gene as expressed in *Escherichia coli* and in potato tubers was compared. The data consist of SDS-PAGE co-migration, Western blot analysis, staining for carbohydrate residues, N-terminal amino acid sequence analysis and biological equivalence against *Leptinotarsa decemlineata*. These data are adequate to support the equivalence of the microbially- and plant-produced protein for use in the toxicology studies.

3. Characterization of the Major Tryptic Fragment from Colorado Potato Beetle Active *Bacillus thuringiensis* subsp. *tenebrionis*. - The purity and activity of a 55kD protein released with tryptic digestion of the *B.t.* Cry III(A) delta endotoxin purified from *E. coli* was shown to have a similar size, immunoreactivity and amino acid sequence to the 55kD fragment found in potato tubers. The 55kD protein had somewhat higher bioactivity than the 68kD full-length delta endotoxin from *B.t.t.*

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These data support the contention that both the 55kD and 68kD forms of the CryIII(A) delta endotoxin found in the plant were similar to those occurring in *B.t.t.*

4. Characterization of Colorado Potato Beetle Active *Bacillus thuringiensis* subsp. *tenebrionis* Protein Produced in *Escherichia coli.* - The method of preparing by fermentation the delta endotoxin from *B.t.t.* in *E. coli* was presented. The protein was characterized for purity and stability after purification. This data indicates that normal fermentation techniques were used to produce the plant equivalent, microbial CryIII(A) delta endotoxin.

5. Compositional Comparison of Colorado Potato Beetle (CPB) Active *Bacillus thuringiensis* subsp.*tenebrionis* Proteins Produced in CPB Resistant Potato Plants and Commercial Microbial Products. - The CryIII(A) delta endotoxin as expressed in potato tissue or an *E. coli* alternative gives a similar immunoreactivity and electrophoretic mobility to registered microbial products producing the same delta endotoxin.

C. TOXICOLOGY ASSESSMENT

Toxicity - The delta endotoxin proteins of *B. thuringiensis* have been intensively studied and no indications of mammalian toxicity have been reported. Furthermore, approximately 176 different *B. thuringiensis* products have been registered since 1961 and the Agency has not received any reports of dietary toxicity attributable to their use. Therefore, the Agency does not anticipate any mammalian toxicity from this protein in plants based on the use history of *B. thuringiensis* products.

The data submitted by Monsanto indicate that this protein would be non-toxic to mammals under the proposed use. Adequate information was submitted to show that the test material derived from microbial cultures was essentially identical to the protein as produced by the potatoes. Production of a microbial Cry III(A) delta endotoxin equivalent to plant-produced delta endotoxin was chosen in order to obtain sufficient material for mammalian testing. In addition, the *in vitro* digestibility studies indicate the protein would rapidly be degraded following ingestion.

The genetic material necessary for the production of the *B.t.* CryIII(A) delta endotoxin are the nucleic acids (DNA and RNA) which comprise the CryIII(A) gene and its controlling sequences. DNA and RNA are common to all forms of life, including plants, and the Agency knows of no instance where these nucleic acids have been associated with toxic effects related to the consumption of food. These ubiquitous nucleic acids as they appear in the subject active ingredient have been adequately characterized by the applicant. Therefore no mammalian toxicity

is anticipated from dietary exposure to the genetic material necessary for the production of the *B.t.* CryIII(A) delta endotoxin in potatoes.

Allergenicity - Despite decades of widespread use of *B. thuringiensis* as a pesticide (it has been registered since 1961), there have been no confirmed reports of immediate or delayed allergic reactions from exposure. Such incidents, should they occur, are required to be reported under FIFRA section 6(a)(2) and as a data requirement for registration of microbial pesticides (40CFR 158.740 and Subdivision M of the FIFRA testing guidelines, NTIS # PB89-211676).

Recent submitted *in vitro* studies also confirm that the delta endotoxin would be readily digestible *in vivo*. Other studies on related proteins are consistent.

Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by heat, acid, and proteases, are glycosylated and present at high concentrations in the food. The delta endotoxins are not present at high concentrations, are not resistant to degradation by heat, acid and proteases, and are apparently not glycosylated when produced in plants. The company has submitted data to indicate that the *B.t.* Cry III(A) delta endotoxin is rapidly degraded by gastric fluid *in vitro*, is not present as a major component of food, and is apparently non-glycosylated when produced in plants.

Based on this information discussed above, the BPPD concludes that this product is not likely to be allergenic.

Submitted Data

1. Acute Oral Toxicity of *B.t.t.* Protein - The *B.t.* Cry III(A) delta endotoxin was determined to be stable and the dosing concentrations were determined to be 74.9 mg/ml, 14.62 mg/ml, and 7.4 mg/ml. *B.t.* Cry III(A) delta endotoxin was not toxic by oral gavage when mice were dosed with up to 5220 mg/kg body weight. These results placed this protein in TOX CATEGORY IV.

2. In-Vitro Digestibility of *B.t.t.* Protein - The 68 kD and 55kD *B.t.* Cry III(A) proteins degraded within 30 seconds in simulated gastric fluid when analyzed by western blot and were not active against Colorado potato beetle after degradation. The 68kD *B.t.* Cry III(A) protein degraded to 55kD within 2 hours of incubation in simulated intestinal fluid. The 55 kD form remained unchanged after 14 hours of incubation and retained its bioactivity and western blot results. These results indicate that, following ingestion by humans, the *B.t.* Cry III(A) proteins will be degraded like other proteins to amino acids and peptides similar to those occurring in a normal human diet.

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D. ENVIRONMENTAL FATE

Based on the data submitted and a review of the scientific literature, EPA concludes that there is no foreseeable risk of unplanned pesticide production through gene capture and expression of the *B.t.* Cry III(A) delta endotoxin gene in wild relatives of the transformed plant, *Solanum tuberosum* L., in potato production areas of the United States. *S. tuberosum* can hybridize naturally with non-tuber bearing *Solanum* species. Only two species of tuber-bearing wild species of *Solanum* occur in the United States, *Solanum fendleri* and *Solanum jamesii*. Although theoretically capable of hybridization with *S. solanum*, both wild species have geographic and genetic limitations that preclude opportunities for such gene exchange to occur.

E. ECOLOGICAL EFFECTS

1. Avian Data - Monsanto conducted two dietary avian toxicity studies using the bobwhite quail and seven different potato lines producing the *B.t.* Cry III(A) delta endotoxin. The studies were both scientifically sound and no treatment mortality, differences in food consumption or behavior was observed between the dosed (50,000 ppm from potato tubers) and control birds. These studies adequately address potential avian toxicity concerns for *B.t.* Cry III(A) delta endotoxin produced in potato and BPPD believes that no additional avian studies should be needed.

2. Aquatic Data - Monsanto did not submit any aquatic studies for this product. Since the *B.t.t.* insect control protein is contained within the potato tissue, exposure to aquatic organisms is considered to be unlikely. Therefore, BPPD believes that aquatic testing is not necessary.

3. B.t.t. Protein Comparison - To ensure that the truncated *B.t.* CryIII(A) delta endotoxin produced in the potato plants will not have an altered host-range of susceptible insects relative to the native full-length protein, comparative insect host-range studies have been submitted by Monsanto. The data consisted of SDS-PAGE co-migration, Western blot analysis, staining for carbohydrate residues, N-terminal amino acid sequence analysis, and biological equivalence. The results demonstrated that the *B.t.* CryIII(A) delta endotoxin with respect to the parameters tested was equivalent to the natural protein.

4. Non-Target and Beneficial Insects - Monsanto submitted three standard non-target insect studies (parasitic wasp, ladybird beetle and green lacewing). The results of these studies indicated that the *B.t.* CryIII(A) delta endotoxin produced in potato plants is practically nontoxic to parasitic hymenoptera (*Nasonia vitripennis*), green lacewing (*Cheysopa carnea*) and lady bird beetle (*Hippodamia convergens*).

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5. Honeybee Toxicity Study - In light of the production of *B.t.* CryIII(A) delta endotoxin protein in pollen and its subsequent exposure to honeybees, Monsanto was required to submit a larval honeybee study. Monsanto also submitted an adult honeybee study which was not required for registration. The adult and larval honeybees were dosed with *B.t.* in a sucrose and honey solution. The testing indicated that there was no significant loss of *B.t.* protein bioactivity in honey or sucrose solutions when maintained for up to 7 days at a approximately 28 C. The adult honeybee study was found to be invalid due to excessive mortality in the controls. Since this study was not required, it will not have to be repeated. The larval honeybee study produced useable results and indicated that *B.t.* CryIII(A) delta endotoxin in potato is practically nontoxic to honeybee larvae. However, the study was not validated with a positive control. This validation may be submitted as a condition of the registration.

6. Mammalian Toxicity - Monsanto submitted a mammalian toxicity study reviewed in the Toxicity Assessment (Section C). The protein was found to be nontoxic to mice. Therefore, BPPD believes that the *B.t.* CryIII(A) delta endotoxin protein should not present a risk to nontarget mammalian species.

7. Nontarget soil organism testing - The registrant did not submit any testing on soil organisms. Because of literature reports describing adverse effects on soil invertebrates from conventional *B. thuringiensis* products and the potential for exposure from *B.t.* CryIII(A) delta endotoxin protein in the plant debris left in the field after harvest that soil organisms will feed upon, these studies will need to be submitted by the registrant. The organisms to be tested are the earthworm and a soil invertebrate, *Collembola* (springtails). Test protocols using *Collembola* and earthworms have been developed and are available from a number of sources. Dr. Gary Reed of Oregon State University reported at the March 1, 1995 SAP subpanel meeting that no effects on *Collembola* were seen in their field study. However, single-species studies should be submitted to confirm the assumption that *B.t.* CryIII(A) delta endotoxin will not affect these organisms.

Ecological Effects Conclusion - This product should not cause adverse effects to: Avian species, wild mammals, or nontarget and beneficial insects. The aquatic testing was waived based on a lack of exposure because the *B.t.* CryIII(A) delta endotoxin protein is contained in the potato tissue. Studies on the toxicity of the active ingredient to springtails and earthworms are necessary as a condition of registration, as is positive control validation of the honeybee larvae study. There are no concerns about ecological effects of the genetic material.

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F. THREATENED AND ENDANGERED SPECIES

It has been determined that the registration will not effect a threatened or endangered species.

One concern is that the production of the endotoxin may spread to wild relatives, which may be food for non-target organisms. The fate analysis predicts that unplanned pesticide production through gene capture and expression of the CryIII(A) gene in wild relatives of the transformed plant, *Solanum tuberosum L.*, or by feral populations in potato production areas of the United States is not foreseeable.

Spray drift exposure to non-target organisms including threatened and endangered species may be a concern for conventional chemicals but is not a concern for this product as the pesticidal protein and the genetic material necessary for its production is produced within potato plants, rather than applied via foliar spray.

The genetic material that are necessary for the production of the CryIII(A) delta endotoxin consist of nucleic acids that are ubiquitous in the environment and would therefore not cause effects on non-target organisms.

Because the delta-endotoxin has only been known to function by a dietary route of exposure, an endangered species or threatened species would have to eat the plant to be exposed.

The known host range of the CryIII(A) delta endotoxin is restricted to Coleopteran species. The submitted non-target insect testing confirm this generalization. There are a number of endangered and threatened species of coleopterans, but it is extremely improbable that they would be exposed to the delta endotoxin found in potatoes because they are not likely to live near potato fields nor would they eat potato plants.

No endangered or threatened avian species feed on potato plants. Even if such feeding should occur, the submitted dietary toxicity study suggests that no effects would result.

No aquatic species are known to feed on potato plants. Therefore exposure to either the *B.t.* CryIII(A) delta endotoxin or the genetic material necessary for its production is extremely improbable.

With the exception of man, few mammals feed on potato plants. Even if such exposure should occur, the results of the submitted acute oral toxicity study indicate no effects on mammalian species will occur.

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G. DEVELOPMENT OF RESISTANCE AND RESISTANCE MANAGEMENT

The Colorado Potato Beetle (CPB) has demonstrated a distinct ability to develop resistance to a wide variety of conventional insecticides. Based on the analysis of available scientific information, the Agency has determined that there is a potential for resistance to develop to the *B.t.* CryIII(A) delta endotoxin produced in potatoes. The development of resistance could contribute to the loss of effectiveness of this plant-pesticide.

Monsanto has developed a voluntary resistance management plan for the *B.t.* CryIII(A) delta endotoxin produced in potatoes. The Agency and the March 1, 1995 SAP subpanel have reviewed the Monsanto resistance management plan and have determined that it is a scientifically-sound and workable resistance management plan to address resistance to the *B.t.* CryIII(A) delta endotoxin produced in potatoes. It includes all of the general elements necessary to reduce the selection pressure on the target pest, CPB, and therefore, reduce the probability for resistance to occur. Monsanto has agreed to voluntarily implement the resistance management plan for the *B.t.* CryIII(A) delta endotoxin produced in potatoes and has agreed to continue to voluntarily work with the Agency on refinements to the resistance management plan as more information is gathered during wide-scale commercial use. However, the Agency realizes that even with a workable resistance management plan, there is still a potential for resistance to occur. Resistance to *B.t.* can also emerge through the use of microbial *B.t.* sprays.

The Agency's analysis, comments provided by the March 1, 1995 SAP subpanel (final report dated March 16, 1995), comments from members of the public, and the Agency's responses to these comments and recommendations on possible future refinements of Monsanto's resistance management strategy for the *B.t.* CryIII(A) delta endotoxin produced in potatoes is summarized in two attachments: (1) May 2, 1995 memorandum entitled "Analysis of SAP and Public Comments on Pesticide Resistance Management for the *B.t.* CryIII(A) delta endotoxin expressed in potatoes and the PRMW recommendations" and (2) December 23, 1994 memorandum entitled "Evaluation of Monsanto document (September 1, 1994) [D207200] 'Strategies to Maximize the Utility and Durability of Colorado Potato Beetle Resistant Potatoes.' " Below is a summary of the Agency's analysis of the SAP comments, oral and written comments to the SAP, additional comments provided following the SAP meeting, and the Agency's conclusions regarding Monsanto's resistance management plan for the *B.t.* CryIII(A) delta endotoxin produced in potatoes.

The SAP subpanel is in agreement with the Agency's review of the Monsanto plan for *B.t.* CryIII(A) delta endotoxin produced in potatoes and the general elements necessary for a resistance

management plan to address resistance to *B. thuringiensis* delta endotoxins produced in potatoes.

Written and oral statements were provided to the SAP on the subject of resistance management of the *B.t.* CryIII(A) delta endotoxin produced in potatoes. All commenters to the SAP, except for the Union of Concerned Scientists, believe that the potential for CPB resistance to the *B.t.* CryIII(A) delta endotoxin produced in potatoes is not an issue which should delay the commercialization of this variety. Information was provided on the validity of the high dose expression strategy, appropriate monitoring, the role of refugia, IPM, and the role of beneficial insect populations. Many commenters noted that there is no evidence that *B.t.* CryIII(A) delta endotoxins produced in plants will necessarily select for resistance more rapidly than *B.t.* toxins deployed in sprays. Commenters noted that no field resistance to foliar *B. thuringiensis* subsp. *tenebrionis* sprays has been detected in North America in an intensive monitoring program involving CPB populations from all production areas.

The Agency has received additional comments following the SAP on pesticide resistance management for *B. thuringiensis* plant-pesticides requesting that EPA refrain from registering Monsanto's *B.t.* CryIII(A) delta endotoxin produced in potatoes until "further information is available on workable resistance management strategies." Several of these commenters expressed concerned that widespread adoption of *B. thuringiensis* plant-pesticides will accelerate the development of resistance and that *B.t.* insecticidal sprays will be rendered ineffective. The Foundation on Economic Trends petitioned the Agency to refrain from registering any plants that have been genetically altered to produce *B. thuringiensis* plant-pesticides. Additional comments were received following the SAP meeting which indicated that the pesticide resistance management strategy developed by Monsanto for the *B.t.* CryIII(A) delta endotoxin produced in potatoes was workable and supported the registration of this product.

The Agency and the SAP agree that Monsanto's resistance management strategy for the potato variety producing the *B.t.* CryIII(A) delta endotoxin, although adequate for the present, should be further refined in the future as additional data become available. Many of the specific questions with respect to monitoring for resistance development and strategies to retard resistance development can best be addressed when use of the potatoes producing the *B.t.* CryIII(A) delta endotoxin has reached commercial scale production over a period of several years throughout potato producing regions. Refinement of resistance management strategies are typically needed during the years of actual use of any pesticide.

The Agency believes that the Monsanto pesticide resistance management strategy should be flexible enough to respond to

additional data gathered on the pest biology and behavior, refugia, and from monitoring to adjust to long-term resistance management strategies. Monsanto has agreed to continue to work voluntarily with the Agency to refine the pesticide resistance management strategy for the *B.t.* CryIII(A) delta endotoxin produced in potatoes. Specifically, areas for further development and refinement include:

1. Continued refinement of information on the reproductive strategies of CPB with respect to gene flow, particularly regarding adult movement, larval movement, behavioral responses including mating studies.

2. Continued refinement of optimal refugia strategies.

3. Continued development of a specific monitoring plan including sites to be sampled, timetable for development, education of growers on sampling for resistance, collecting specimens to evaluate for resistance, and providing specific recommendations on how to eradicate resistant individuals to prevent survival of a resistant population.

4. Continued development of a data base to monitor the use of the genetically modified potatoes and correlate possible resistant reports with the use sites.

5. Development of a discriminating dose assay.

6. Continued development of educational materials, in addition to the technical bulletin, for growers on how to use genetically modified potatoes, monitor for resistance, and when to use other insecticides.

7. Continued refinement of IPM recommendations at the local level such as crop rotation.

8. Continued development of novel CPB control mechanisms with different modes of action.

IV. BENEFITS

A. TARGET PEST: COLORADO POTATO BEETLE

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), is the most damaging pest of the North American potato crop (Casagrande 1987). Both adult and larval stages can severely defoliate potatoes and reduce tuber yields to levels which have become a limiting factor in continuing potato production in some areas (Hare 1980, Ferro et al. 1983, Shields and Wyman 1984). This pest is most severe in the eastern and Midwest potato production areas where control costs often exceed $200 per acre and is becoming an increasing problem in the

Northwest (Casagrande 1987, Ferro and Boiteau 1992). Currently, growers depend primarily upon chemical pesticides to control this pest. It is estimated that approximately one million pounds of chemical insecticide active ingredients are applied annually (USDA 1993). Foliar microbial formulations of *B.t.* and non-chemical control practices including crop rotation, propane flaming, vacuum suction, trap cropping, and trenching are available; however, these methods are not always effective, economical or practical and chemical insecticides remain the treatments of choice. Esfenvalerate, carbofuran, azinphos-methyl, endosulfan, permethrin, and disulfoton are the most commonly used chemical active ingredients applied for CPB control.

The CryIII(A) delta endotoxin produced in potatoes is identical to that found in nature and in commercial *B.t.* formulations such as Foil (EPA Reg No. 55638-10) or M-One (EPA Reg No. 53219-1). However, these potatoes produce the Cry III(A) delta endotoxin throughout the plant for the length of the growing season at a level sufficient to control all life stages of the CPB. In contrast, the application of foliar *B.t.t.* must be frequent and carefully timed to adequately protect the crop as the *B.t.t.* in these formulations degrades quickly in sunlight, washes off easily in the rain, and has limited effectiveness against the most damaging life stages (third and fourth instar larvae) of the CPB.

Field experiments conducted at more than 30 locations throughout the U.S. potato growing region since 1991, have demonstrated that *B.t.t.* potatoes are protected season long from all CPB lifestages. Growers who use *B. thuringiensis* plant-pesticides do not require chemical insecticide applications to control CPB. The Long Island, New York potato production area has CPB populations which are highly resistant to most chemical insecticides. *B. thuringiensis* plant-pesticides produced by potatoes were tested on Long Island provided excellent, season long control of all stages of CPB and high yields without relying on other chemicals for control of CPB.

Because of its severity, the CPB has been subjected to intensive insecticide use, both to control economically damaging densities and as insurance to prevent CPB populations from getting out of control. This in turn has contributed to some of the worst insecticide resistance problems of any pest in the U.S. In many areas, there are no currently registered insecticides that will control the adult beetles effectively. Even with intensive use of chemical pesticide control measures, estimated yield losses of 30 percent have been documented from control failure due to insecticide resistance. The failure rate of new insecticides seems to occur at a rate of about once every 3 years. It is common for growers to spend more than $200 per acre for insecticides to control the potato beetle. Given the large

investment in a potato crop (e.g., $1500 per acre) and the potential gross return (about $3000 per acre). Therefore, these costs have not significantly inhibited insecticide use. Use of potatoes producing the *B.t.* Cry III(A) delta endotoxin may provide environmental benefits by reducing the total amount of chemical pesticides used to control CPB. Use of these potatoes increases the ability of parasites and predators of other potato pests such as aphids and fleahoppers to attain population levels that are effective in regulating these pests.

CPB populations in the major Russet Burbank potato growing regions (Northwest United States) are not yet highly resistant to chemical insecticides and are adequately controlled by available methods. However, CPB infestations are getting steadily worse in these growing regions and *B.t.* Cry III(A) delta endotoxin produced in potatoes will allow growers in these regions to control CPB season long without the use of broad spectrum chemical insecticides.

Biological regulation of aphid species is also a key consideration in management for aphids on potatoes. The green peach aphid can become highly resistant to insecticides following exposure to as few as 2-3 insecticide applications through increased production of carboxylesterase (Devonshire and Moores 1982). This esterase-based resistance has severely reduced the ability of growers to manage *Myzus persicae* with insecticides worldwide and increased the potential for virus transmission in potatoes. The elimination of CPB spray programs (which also pre-select resistance in *M. persicae*) and the increase in effectiveness of biological regulation (which can reduce and in some cases may eliminate the need for insecticidal aphid control) are predictable results of the introduction of CPB-resistant potatoes.

B. CONCLUSIONS ON BENEFITS

Bacillus thuringiensis CryIII(A) delta endotoxin produced in potato has the potential to greatly reduce pesticide exposure to humans and the environment. Agricultural field workers who would be exposed to conventionally applied pesticides through mixing, loading, and applying the pesticide, or exposed through working in fields previously treated, would have almost no exposure to a pesticide which is produced inside the potato in low amounts.

Furthermore, the application of nonspecific chemical insecticide alternatives adversely affect populations of nontarget beneficial arthropods found living in the complex environment of a potato field. Many of these beneficial arthropods are important integrated pest management controls (IPM) for secondary pests such as aphids and leafhoppers. The overall result is that the number of insecticide applications for aphid and leafhopper control will be reduced in most cases in

addition to the reduction in the use of chemical pesticides required for the control of CPB. As of this writing, there have been no identified adverse effects of delta endotoxin to nontarget beneficial arthropods, whether they are parasites, predators, or pollinators.

The Agency has examined the documents supporting the registration and exemption from the requirements of a tolerance for the plant-pesticide *Bacillus thuringiensis* subsp. *tenebrionis* Colorado potato beetle (CPB) control protein and finds that the amended registration will provide numerous benefits including the following: 1) *B. thuringiensis* plant-pesticides reduce the use of conventional chemical pesticides in potato production. No foliar chemical insecticides are needed for CPB control because *B. thuringiensis* plant-pesticides provide season-long control; 2) *B. thuringiensis* plant-pesticides produced by potatoes are more efficacious for CPB control than currently registered pesticides and non-chemical alternatives; 3) avoidance of risks that conventional chemical pesticides have including accidental release or exposure during shipping, storage, mixing and loading, application, and container disposal; 4) protection of ground water quality in potato growing regions which have soil types predisposed to leaching; 5) nontoxic to non-target organisms; 6) Potential reduction of input costs for large and small farms alike; and 7) enhancement of Integrated Pest Management (IPM) programs through increased biological control.

In summary, production of potatoes containing *B.t.* CryIII(A) delta endotoxin has the potential to provide a commercial crop of potatoes requiring fewer pounds and fewer applications of chemical pesticides, thereby saving grower costs and reducing risks to humans and the environment.

V. OTHER CONSIDERATIONS

USDA/APHIS has made a determination that Monsanto's potato is no longer a regulated article. FDA has concluded its consultation with Monsanto on the potato and thereby completed their assessment.

VI. CONDITIONS OF REGISTRATION

The Agency is requiring studies on the effects of the CryIII(A) delta endotoxin on springtails and earthworms. Available field data on this product have not indicated adverse effects to non-target invertebrates. Therefore, while confirmatory data are necessary, no adverse impacts on these organisms are anticipated.

Validation of the honeybee larval toxicity study is also being required as a condition of registration. As stated prior, the larval toxicity study provided acceptable results but needs

to be validated with a positive control. The results of the study indicate no likelihood of adverse effects on this organism.

Agency is requiring the submission of these studies and information within 6 months of the date of registration. Monsanto has agreed to the submission of this information within the specified timeframe.

VII. CONCLUSIONS ON RISKS AND BENEFITS

Pursuant to FIFRA section 3(c)(7)(B), EPA may conditionally amend the registration of a pesticide to permit an additional use if two criteria are fulfilled: 1) the applicant has submitted satisfactory data pertaining to the proposed new use; and 2) amending the registration in the manner proposed by the applicant will not significantly increase the risk of any unreasonable adverse effect.

A. ADDITIONAL USE REGISTRATION UNDER FIFRA 3(c)(7)(B)

Monsanto has submitted satisfactory data pertaining to the proposed additional use which have been described in the preceding sections of this memorandum. The human health effects data are considered complete and no potential adverse effects are foreseen. However, acceptable generic studies on honeybee larvae, *Collembola* and earthworm have not been generated and are conditionally required. The likelihood of risk to these organisms is considered remote given the submitted information and battery of public knowledge concerning the species range of toxicity for the *B.t.* CryIII(A) delta endotoxin, especially within the time allowed for generation and review of the data.

In addition, the second FIFRA criteria has been satisfied as a review of these data and other information indicates that the proposed additional use does not "significantly increase the risk of any unreasonable adverse effect." In essence, this provision requires a determination that the proposed additional use of this product for food and feed, without the limitations imposed in the initial registration, would not modify the cost/benefit ratio so as to cause unreasonable adverse effects taking into account the economic, social, and environmental costs and benefits of this additional use.

The Agency has reviewed the submitted data and other relevant information relative to the amendment of the existing limited registration to permit the unrestricted food or feed use. Based upon this material, the Agency can foresee no unreasonable adverse effects to man or the environment as a result of the new use.

No risks to human health have been identified by BPPD, by the SAP or in the public comments received.

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There are also no risks to avian or aquatic species, wild mammals, or nontarget and beneficial insects. The risk to non-target soil organisms in the period between registration of the new use and the receipt and review of the required data by the Agency is considered remote. There will be only 6 months between the time of registration and the submission of these studies. Also, much of U.S. potato crop has already been planted this year, and only a limited additional acreage of plant-pesticide containing potatoes are likely to be added to this acreage. Given the timing of the data requirements and the planting considerations for potatoes as an agronomic crop, little potential for adverse effect to non-target organisms is foreseen.

The development of pest resistance to the plant-pesticide is a potential cost of the amended registration as it is for many other pesticides. Such resistance could reduce the utility of the existing *Bacillus thuringiensis* subsp. *tenebrionis* and *San Diego* products which also use the CryIII(A) delta endotoxin as sprayable formulations to control the early larval instars of the Colorado potato beetle. Development of resistance to *B.t.t.* could result in the use of more hazardous alternatives at or perhaps slightly above that used today. Foliar sprays of *B.t.t.* are a very small part of the market for CPB control. If *B.t.t.* sprays were no longer effective, organic potato growers would likely rely on cultural and mechanical methods such as crop rotation, trapping, etc.

If Monsanto implements its voluntary resistance management plan as agreed, there will be a reduced probability that resistance will develop. The plan also increases the probability that resistance will be detected and managed should it occur. Monsanto has agreed to implement this plan on a voluntary basis, and cooperate with the Agency in refining various aspects that can only be completed based upon wide-scale commercial use. The SAP has reviewed Monsanto's plan and agrees with the Agency that it is a workable resistance management plan. The Agency encourages users of *B.t.t.* sprays also to practice resistance management.

The potential benefits of the new use have been discussed in more detail in the previous section of the document. The new use offers significant improvement in the control of the CPB over what is available for commercial potato production today. The new use also provides the potential for reduced cost to growers by eliminating application costs. Human health risks (both acute and chronic effects) now associated with the application of more hazardous organophosphate, carbamate, and pyrethroid insecticides for this pest are reduced. Environmentally, the potential adverse effects of organophosphate, carbamate and pyrethroid insecticides will be abated. In addition, use of this plant-pesticide will fit well into existing IPM program which emphasize crop rotation and the use of beneficial insects.

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In summary, when the costs of this proposed use are weighed against the potential benefits, there is no significant increase in the risk of unreasonable adverse effects. While there are some increased costs associated with the proposed additional use of this product associated with the possibility that resistance will develop and the remote chance of short-term effects on non-target soil organisms, these costs continue to be outweighed by the potential economic, health, and environmental gains of use.

B. MODIFICATION OF EXISTING FIFRA 3(c)(5) REGISTRATION

Monsanto has an existing registration granted pursuant to FIFRA section 3(c)(5) that authorizes use of the plant-pesticide to produce seed potatoes. The existing registration is subject to a variety of terms and conditions that limit the scope and duration of the use. The limited scope and duration of that registration allowed the Agency to determine that the pesticide could be registered under FIFRA section 3(c)(5) even though the science data review was not complete, the tolerance exemption petition was still under review, and the SAP report had not been analyzed completely. Those steps are now complete. For the reasons discussed in section VI, B above, BPPD has concluded that additional generic data on the risks to nontarget soil organisms must be submitted but that the pesticide does poses no unreasonable adverse affects.

VIII. RECOMMENDATION

The submitted data in support of this amended registration under section 3(c)(7)(B) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) have been reviewed and determined to be adequate. Studies and information regarding the effects of this product on *Collembola*, earthworms, and honeybee larvae are conditions of registration. The conditional data requirements will confirm the information already reviewed by the Agency regarding effects to certain non-target invertebrate organisms. While resistance to the CryIII(A) delta endotoxin by the Colorado potato beetle may occur, Monsanto has agreed to voluntarily implement a resistance management plan. Amending the existing registration will not cause an increase in significant adverse effects to man or the environment, either as a result of exposure to non-target organisms or from the potential for the development of resistance.

Furthermore, the benefits of the new use pattern have been well established and outweigh potential risks, either from potential adverse impacts to earthworms and collembola or from the development of resistance by the Colorado potato beetle.

Therefore, Biopesticides and Pollution Prevention Division recommends that Monsanto's plant-pesticide product containing the new active ingredient *Bacillus thuringiensis* CryIII(A) delta-

endotoxin and the genetic material necessary for its production in potatoes be CONDITIONALLY REGISTERED under 3(c)(7)(B) of FIFRA for all uses in potato.

CONCUR: _____

NONCONCUR: _____

DATE: 5/5/95